NHS Highland

Scottish Microbiology Reference Laboratory: Lyme disease and tick-borne infections

User manual

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1. Introduction

The Scottish Lyme Disease and Tick-borne Infections Reference Laboratory (SLDTRL) is provided by NHS Highland at Raigmore Hospital, Inverness. The aim of SLDTRL is to provide comprehensive and standardised testing for Lyme disease and other tick-borne infections and to improve the epidemiological data provided to Public Health Scotland (PHS).

Lyme disease is caused by bacteria from the *Borrelia burgdorferi* sensu lato complex. In the UK the bacteria is transmitted to humans through the bite of infected, hard bodied, *Ixodes ricinus* ticks. *Borrelia miyamotoi* disease, which presents as a relapsing fever, can also be transmitted by *Ixodes ricinus* ticks. It is an emerging disease in Europe caused by *B. miyamotoi* bacteria, which are from the relapsing fever group of borrelia, genetically distinct from those that cause Lyme disease. Human granulocytic anaplasmosis (HGA), also an acute febrile illness transmitted by Ixodid ticks, is an infection caused by the bacterium *Anaplasma phagocytophilum*. Tick-borne encephalitis is a rare but emerging human viral disease in the UK, involving the central nervous system. Again, the TBE virus can be transmitted by infected *I. ricinus* ticks.

In addition to serological testing for antibodies to *B. burgdorferi* (Lyme disease), we offer a pan-borrelia PCR on request. This can detect *B. miyamotoi* as well as the Lyme disease causing *B. burgdorferi*. Molecular testing for *Anaplasma phagocytophilum* is also available. Testing for tick-borne encephalitis (TBE) is not yet available at SLDTRL but sera can be sent to the Rare and Imported Pathogens Laboratory (RIPL) at Porton Down for TBE serology.

The NHS Highland Microbiology Department is accredited by the United Kingdom Accreditation Service (UKAS). UKAS Medical accreditation number 9612 (Accredited to ISO 15189:2012). A full list of tests in scope is available on the laboratory homepage. Please note that our anaplasma PCR assay is currently out of scope. Our schedule of accreditation may also be found on the **UKAS website**.

1.1 Contact details

Scottish Lyme Disease and Tick-borne Infections Reference Laboratory
Microbiology Department
Zone 3, Raigmore Hospital
Old Perth Road
Inverness
IV2 3UJ

Telephone (Monday to Friday, 9am to 5pm): 01463 704206 (direct)
Email: nhsh.smirl@nhs.scot
DX address: DX6180102 - 90IV

1.2 Key personnel

The staff may be contacted at any time for advice and support. Complaints should be directed to Director/Clinical Lead.

Principal Clinical Scientist (Director):	Dr Sally Mavin
Clinical leads:	Dr Mairi Cullen & Dr Alex Cochrane
Microbiology Service Manager:	Dr Joanne Smullen

2. Opening hours

Core Hours are Monday to Friday, 9am to 5pm.
SLDTRL does not operate an out-of-hours service

3. Service provided

3.1 Samples and turnaround times

Samples and turnaround times

Test	Specimen required	Volume	Turnaround time	Comment
Serology Screening IgG/IgM CLIA & confirmatory IgG and IgM Immunoblot for *B. burgdorferi* antibodies	Clotted blood	5ml	Screen: 3 days Immunoblot: 5 days	Testing **not** indicated for asymptomatic patients or patients with Erythema Migrans (EM)
	Serum	500µl		
CSF testing[a,b] Serology to identify intrathecal production of *B. burgdorferi* antibodies and CXCL13 testing	CSF **and** paired serum	1ml 500µl	14 days (currently albumin and IgG levels cannot be done in-house and are referred)	CSF samples **must** be accompanied by serum taken on the same day. CSF must not be bloodstained. Please provide CSF white cell counts
Pan-borrelia PCR *B. burgdorferi* (Lyme disease)	Joint fluid	200µl	7 days	Samples for PCR **must** be accompanied by serum sample
	Tissue	25 mg		
B. miyamotoi (relapsing fever)	EDTA whole blood (unspun)	5ml	7 days	Sample should be taken during febrile phase (<4 weeks onset)
	CSF	200µl		

Test	Specimen required	Volume	Turnaround time	Comment
PCR for *Anaplasma phagocytophilum*	EDTA whole blood (unspun)	5ml	7 days	Sample should be taken in acute phase of illness (<4 weeks onset)

[a]CSF testing by PCR not routinely offered for patients with suspected neuroborreliosis due to poor sensitivity. CSF serology is the method of choice

For urgent requests please contact the laboratory.

3.2 Laboratory tests

3.2.1 *B. burgdorferi* (Lyme disease) serology

All samples are tested using a sensitive screening assay for detection of IgG and IgM antibodies and reactive/ equivocal samples confirmed by IgG and IgM immunoblots.

3.2.2 *B. burgdorferi* CSF testing

Paired serum and CSF samples (i.e. taken on the same day) are required for the analysis of CSF antibodies.

B. burgdorferi antibodies may be detected in CSF earlier than in serum. This is indicative of acute neuroborreliosis. However, laboratory confirmation of neuroborreliosis is generally based on demonstrating intrathecal synthesis of *B. burgdorferi* antibodies, usually in the presence of pleocytosis. This is done by calculating the antibody index, which incoporates *B. burgdorferi* antibody levels as well as albumin and IgG concentrations in both the paired CSF and serum. An antibody index of >1.5 is indicative of intrathecal antibody production.

Currently, determination of albumin and IgG levels cannot be done in-house and has to be sent to an external laboratory. Provision of these concentrations by the referring laboratory would ensure a more timely result.

Please provide CSF cell counts if available.

CXCL13 is a chemokine that has been shown to be highly elevated in patients with neuroborreliosis. CXCL13 production is rapid, meaning earlier diagnoses may be possible. The level of CXCL13 has also been shown to decrease following successful antibiotic treatment and resolution of disease. Please note: CXCL13 can also be raised in other conditions such as HIV and neurosyphilis.

3.2.3 Pan borrelia PCR

3.2.3.1 *B. burgdorferi* (Lyme disease)

Samples for PCR (joint fluid/tissue) **must** be accompanied by a serum sample. If a patient with suspected Lyme arthritis is seronegative for *B. burgdorferi* Lyme arthritis is unlikely, therefore PCR testing of joint fluid is not indicated and sample will not be tested. Please note: a negative PCR result does not exclude the diagnosis of Lyme disease as the sensitivity of PCR varies greatly between sample type and disease stage.

3.2.3.2 B. miyamotoi

Whole blood samples (EDTA) are the most suitable sample type for PCR due to the relatively high level of bacteraemia compared to that observed for Lyme disease patients. Samples should be taken during the febrile phase (bacteraemia rare between relapses). For patients with suspected meningoencephalitis, CSF samples should be considered.

3.2.4 *A. phagocytophilum* PCR

Whole blood samples (EDTA) should be taken within the acute phase of the illness (first 4 weeks). Samples will not be tested if no date of onset stated or onset >4 weeks.

3.3 Specialist advice

The laboratory can be contacted by medical practitioners for clinical enquiries regarding Lyme disease and other tick-borne infections as well as advice regarding specialist testing.

We cannot offer clinical advice directly to patients.

4. Clinical Information and testing/referral criteria

This is vitally important in deciding how to test a sample and to provide appropriate interpretative comments on results to users. Please give specific symptoms and signs and details of any tick bite/exposure (see SLDTRL request form, Section 12.).

Testing is not indicated for asymptomatic patients with a tick bite or patients with Erythema migrans (EM).

4.1 Early Lyme borreliosis

- Onset of symptoms usually 3 to 30 days following a tick bite/exposure.
- Erythema migrans (EM), which is classified as a >5cm spreading rash from a tick bite, is diagnostic but the rash may be atypical or absent. Flu-like symptoms: tender muscles/ joints, pyrexia and lymphadenopathy can also occur.
- Meningitis/ encephalitis are rare.
- In patients with neuroborreliosis lymphocytic pleocytosis (i.e. WBC>100, 90% lymphocytes) is usually observed in the CSF with mild to moderately elevated CSF protein. CXCL13 is usually highly elevated.
- **Patients with a tick bite and EM should be treated empirically. Testing is not indicated unless there is doubt about the diagnosis.**
- For patients with suspected early Lyme borreliosis a negative result does not exclude the possibility of *B. burgdorferi* infection. Failure to detect antibodies is most likely to be due to sample collection prior to detectable antibody development. Antibody levels can take up to 10 weeks to develop, thus if Lyme disease remains a possibility the test should be repeated.

4.2 Late Lyme borreliosis

- Weeks to months after tick exposure.
- Multiple or single system involvement of skin, joints, heart, brain or peripheral nerves.
- SLDTRL will test EIA negative samples from patients with onset of symptoms >12 weeks by immunoblot (as per NICE guidelines) **only** when referred by an appropriate specialist physician, such as infectious disease consultant, GP has spoken to the reference laboratory directly or if there are discrepant results on testing by a second laboratory.

4.3 *B. miyamotoi* disease

- Acute, often cyclical, febrile illness after tick bite (up to 40 days, Jiang et al, 2018).
- Fever is often accompanied by headache, fatigue, muscle and joint aches and nausea.
- Meningoencephalitis in immunocompromised patients has been described but is rare.
- Rash is very uncommon (8% cases, Molloy et al. 2015)

- Other laboratory findings include leucopenia, thrombocytopenia, mildly raised transaminases (ALT, AST). In patients with meningoencephalitis mononuclear pleocytosis may be observed in the CSF with raised protein.

4.4 Anaplasmosis

- Acute febrile illness after tick bite (incubation period of 5 to 21 days)
- Severe headache, generalised myalgia/arthralgia and less commonly nausea, vomiting and cough/difficulty breathing have been observed.
- Rash (erythematous, non-pruritic) is rare.
- Other laboratory findings include thrombocytopenia (90%), leucopenia (70%), raised CRP (almost all patients), markedly raised transaminases (ALT, AST).

4.5 Tick-borne encephalitis (TBE)

- Disease course often biphasic:
    - First viraemic phase (lasting 2-10 days, mean 5 days): Fever, tiredness, headache, muscle pain and nausea (usually 7 days but up to 28 days following tick bite)
    - Second phase (following asymptomatic interval lasting 1-33 days, mean 7 days): CNS involvement (meningitis, meningoencephalitis, myelitis, paralysis, radiculitis

  Clinicians should consider the diagnosis of tick-borne encephalitis (TBE) in any person who has appropriate clinical features including meningitis, encephalitis or other appropriate neurological presentation and in whom:

  a. An exposure to ticks is confirmed or probable given history of activities or occupation

  or

  b. No known exposures, but no other cause can be found once more common causes of meningitis and encephalitis have been excluded and infectious cause is considered as part of the differential.

- Refer serum sample (min 0.1ml) for TBE testing to **Rare and imported pathogens laboratory (RIPL) at Porton Down**. If serum samples are suggestive of TBE infection further samples such as CSF or Urine will be requested by RIPL.

5. Referral criteria

5.1 Lyme disease serology

A 5ml clotted blood sample (or 500µl serum) from patients who present with symptoms consistent with Lyme disease (excluding recognised erythema migrans, see below) after tick bite/exposure should be sent to the reference laboratory for serological testing (see Section 3.1 for sample details).

Samples from patients who present with the following clinical details **should not** be referred:

Clinical details: Asymptomatic tick bite/tick bite only
Advice: Seek medical advice if become symptomatic.

Clinical details: Erythema migrans*
Advice: Treat empirically (as per NICE guidelines).

Clinical details: Post treatment
Advice: Monitor clinically. Serology cannot be used to assess treatment success.

Clinical details: No clinical details
Advice: Results for samples without clinical details cannot be interpreted.

* If there is doubt about the presentation of erythema migrans then refer sample for testing.

6. Specimen and request form labelling

For the safety of patients and staff, the NHS Highland Area Laboratory Service operates a strict specimen acceptance policy (full copy is available on request).

Specimens may be submitted either using a referring laboratory's own request form or with the (SLDTRL) request form. However, both the request form and sample must be labelled with a minimum of three pieces of information to allow unequivocal identification of the patient.

Minimum data set

Request form	Sample
Patient's surname[1]	Patient's surname[1]
Patient's forename(s)	Patient's forename(s)
CHI number[2]	CHI number[2]
Date of birth (not age)	Date of birth (not age)

[1]Or accepted coded identifier (e.g. soundex code, NaSH number)

[2] Where the CHI number is not available a third point of identification (e.g. address) must be provided.

In addition, please ensure the request form includes:

- name and location of sender (or details of where the final report should be sent if different)
- specimen type
- date and time of collection
- associated clinical information

Specimens that do not conform to the minimum data set will **not** be processed by the laboratory.

The department will reject specimens that present a health and safety hazard to staff (e.g. leaking specimens, contamination of specimen containers external surfaces), inappropriate and insufficient specimens.

7. Specimen transportation

- Samples must be appropriately packaged and transported in accordance with current regulations.
- If unsure of the current regulations please contact the laboratory for advice.
- Please ensure that packages contain sufficient absorbent material to contain all liquid.
- Please ensure request forms are placed between the plastic container and cardboard outer and not with the sample inside the plastic container.
- Samples should be sent to the laboratory via Royal Mail or DX courier to the address shown in section 1.1.
- NHS Highland users should use appropriate transport within NHS Highland and should refer to the NHS Highland transport policy on the intranet about specifications for delivery.

8. Charges

SLDTRL is funded by National Services Scotland (NSS) and testing is carried out free of charge for Scottish NHS laboratories. Samples received from other laboratories and private companies will be subject to charge – prices are reviewed annually and are available on request.

9. Results

Results are emailed to the referring laboratory or can be obtained from Sci-store (NHS Highland and Health boards with store to store access). Significant results or those that are required urgently will be reported by telephone.

10. Treatment

Information on treatment of Lyme borreliosis can be found in the **NICE guidelines** and, for NHS Highland users, the **NHS Highland formulary**.

Doxycycline (oral, 100mg twice daily) for 14 days is recommended as the treatment of choice for *B. miyamotoi* disease (CDC, Krause *et al* 2015, Gugliota *et al* 2013). IV ceftriaxone should be considered for meningoencephalitis (14 to 28 days if meningoencephalitis, 28 days if immunocompromised) (Barbour *et al* 2021.

Doxycycline is recommended as the treatment of choice for anaplasmosis (oral 100mg twice daily for 10 days, Biggs *et al* 2016). Treatment should not be delayed for laboratory results. If anaplasmosis is clinically suspected EDTA whole blood should be taken and the patient given antibiotic treatment.

There is no specific antiviral therapy for TBE. Treatment relies on supportive management.

11. Prevention

When visiting areas inhabited by ticks, regular checks should be made and any ticks removed as soon as possible (and within 24 hours). An acaricide or tick repellent may also be used. Light coloured clothing makes it easier to spot ticks before they may attach. Find out more on **NHS Inform**.

12. SLDTRL request form

A copy is available on the **NHS Highland website**.

13. Online references

2. NICE guidelines
3. NHS Inform for prevention
4. NHS Highland website
5. NHS Highland intranet (available to NHS Highland only)
6. Treatments and Medicines App
7. Factsheet on anaplasmosis (CDC)
8. Factsheet on Tick-borne relapsing fever (ECDC)
9. UpToDate factsheet – Borrelia miyamotoi
10. Factsheet on Tick-borne encephalitis (ECDC)

14. Articles

- Biggs HM, Behravesh CB, Bradley KK *et al* (2016) Diagnosis and management of tickborne rickettsial disease: Rocky Mountain spotted Fever and other Spotted Fever Group Rickettsioses, Ehrlichioses, and Anaplasmosis – United States. MMWR 65(2); 1-44.
- Molloy PJ, Telford SR 3rd, Chowdri HR, Lepore TJ, Gugliotta JL, Weeks KE, Hewins ME, Goethert HK, Berardi VP (2015) *Borrelia miyamotoi* disease in the northeastern United States: A case series. Annals Internal Medicine 163(2):91-8.
- Gugliota JL, Goethert HK, Berardi VP (2013) Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. N Engl J Med 368(3):240-5.
- Jiang B, Hia N, Jiang J *et al* (2018) *Borrelia miyamo*toi Infections in Humans and Ticks, Northeastern China. Emerg Infect Dis 24(2):236.
- Krause PJ, Fish D, Narasimhan S, Barbour AG (2015) Borrelia miyamotoi infection in nature and in humans. Clin Microbiol Infect 21(7):631-9.

15. Laboratory diagnosis of Lyme borreliosis algorithm